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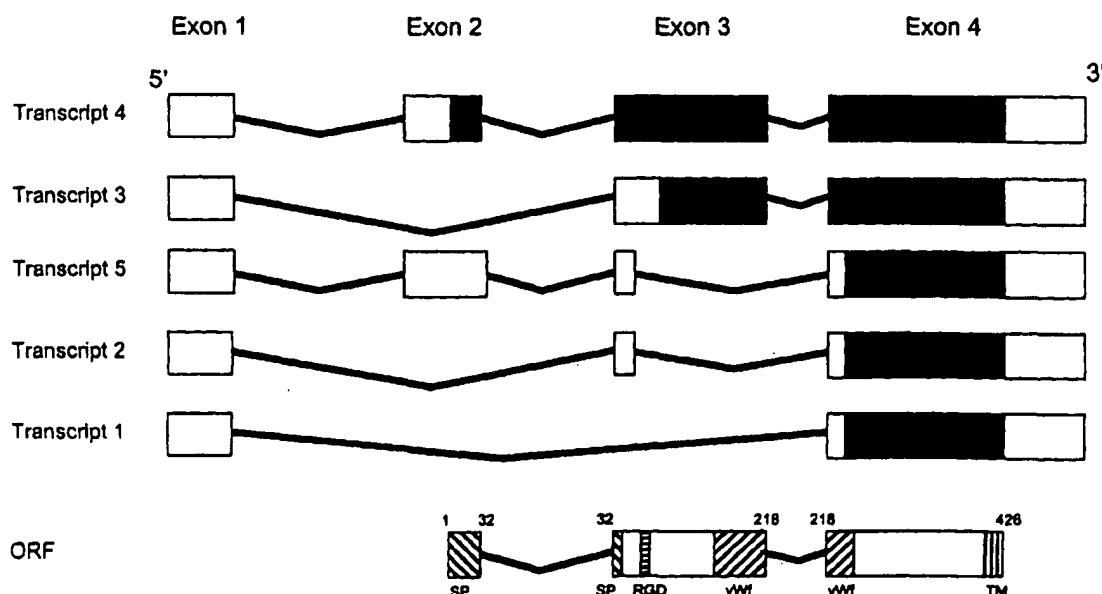
[CA/US]; 48 El Cerrito, San Anselmo, CA 94960 (US). **MACDONALD, Marcla, L.E.** [CA/CA]; 102-1345 West 13th Ave., Vancouver, British Columbia V6H 1N7 (CA). **FRANCHINI, Patrick** [CA/CA]; 49-7345 Sandborne Ave., Burnaby, British Columbia V3N 4W6 (CA). **GOLDBERG, Yigal, Paul** [CA/CA]; 62 West 18th Avenue, Vancouver, British Columbia V5Y 2A4 (CA). **KAMBOJ, Rajender, K.** [CA/CA]; 5809 Sperling Avenue, Burnaby, British Columbia V5E 2T6 (CA). **PAPANIKOLAOU, George** [GR/GR]; 1st Dept of Internal Medicine, National and Kapodistrian University of Athens, 6 Chr. Lada str., GR-10561 Athens (GR).

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[Continued on next page]

(54) Title: **JUVENILE HEMOCHROMATOSIS GENE (HFE2A), EXPRESSION PRODUCTS AND USES THEREOF**



(57) Abstract: Polynucleotide and polypeptide sequences for HFE2A, as well as mutations associated with juvenile hemochromatosis, and methods of utilizing these for the screening and identification of agents for the treatment of diseases of iron metabolism, including small organic compounds, are disclosed along with methods of treating and/or ameliorating diseases of iron metabolism, especially in human patients are disclosed. Diagnostic compounds, kits and methods using HFE2A are also described.



(84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

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6 May 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/47 G01N33/50 A61K35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, Sequence Search, BIOSIS, EMBASE, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 02/051438 A (MAX PLANCK GESELLSCHAFT ; STAHL BERND (DE); MACCHI PAOLO (DE); BONHOEF) 4 July 2002 (2002-07-04) the whole document & DATABASE EMBL retrieved from EBI accession no. AX470382 abstract ----- -/--	1-13,84



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *A* document member of the same patent family

Date of the actual completion of the international search

17 January 2005

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17. 02. 2005

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>TAUPIN J-L ET AL: "Functional quantification of cyclosporine A and FK506 in human whole blood by flow cytometry, using the green fluorescent protein as an interleukin-2 reporter gene"</p> <p>JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol. 256, no. 1-2, 1 October 2001 (2001-10-01), pages 77-87, XP004300784</p> <p>ISSN: 0022-1759</p> <p>the whole document</p>	1-13,84
Y	<p>AFANASSIEV VICTOR ET AL: "Application of yeast cells transformed with GFP expression constructs containing the RAD54 or RNR2 promoter as a test for the genotoxic potential of chemical substances"</p> <p>MUTATION RESEARCH, vol. 464, no. 2, 24 January 2000 (2000-01-24), pages 297-308, XP002302121</p> <p>ISSN: 0027-5107</p> <p>the whole document</p>	1-13,84
P,X	<p>AMERICAN JOURNAL OF HUMAN GENETICS, vol. 73, no. 5, November 2003 (2003-11), page 205, XP009038371</p> <p>53RD ANNUAL MEETING OF THE AMERICAN SOCIETY OF HUMAN GENETICS; LOS ANGELES, CA, USA; NOVEMBER 04-08, 2003</p> <p>ISSN: 0002-9297</p> <p>the whole document</p>	1-13,84
Y	<p>GONZALEZ JESUS E ET AL: "Intracellular detection assays for high-throughput screening"</p> <p>CURRENT OPINION IN BIOTECHNOLOGY, vol. 9, no. 6, December 1998 (1998-12), pages 624-631, XP000874867</p> <p>ISSN: 0958-1669</p> <p>the whole document</p>	14,62-66
Y	<p>HERTZBERG R P ET AL: "High-throughput screening: New technology for the 21st century"</p> <p>CURRENT OPINION IN CHEMICAL BIOLOGY, CURRENT BIOLOGY LTD, LONDON, GB, vol. 4, no. 4, August 2000 (2000-08), pages 445-451, XP002256444</p> <p>ISSN: 1367-5931</p> <p>page 447, column 2 - page 448, column 1</p>	14,62-66

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>MATTHEAKIS L C ET AL: "Expression of Cre recombinase as a reporter of signal transduction in mammalian cells" CHEMISTRY AND BIOLOGY, CURRENT BIOLOGY, LONDON, GB, vol. 6, no. 11, November 1999 (1999-11), pages 835-844, XP002265769 ISSN: 1074-5521 the whole document</p>	14,62-66
A	<p>KOLLER E ET AL: "Elucidating cell signaling mechanisms using antisense technology" TRENDS IN PHARMACOLOGICAL SCIENCES, ELSEVIER TRENDS JOURNAL, CAMBRIDGE, GB, vol. 21, no. 4, April 2000 (2000-04), pages 142-148, XP004196016 ISSN: 0165-6147</p>	14,62-66

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2004/000522

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 33-37, 44-48, 50-54, 84-85 (completely) 38-43, 49 (partially)
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 33-54, 82, 83 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: 33-37, 44-48, 50-54 (completely), 38-43, 49 (partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
1-14, 62-66, 84
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-13, 84

Method for identifying a modulating agent for HFE2A gene expression comprising testing a test compound with a genetic construct comprising a reporter gene linked to the HFE2A promoter; Method for producing test data using said method.

2. claims: 14, 62-66

Method for identifying a modulating agent for HFE2A gene expression comprising testing a test compound with a cell expressing an HFE2A gene.

3. claims: 15-32, 85

Method for identifying a modulating agent for HFE2A activity comprising contacting a test compound with the HFE2A polypeptide; Method for producing test data using said method.

4. claims: 33-54

Method of treating a disorder comprising administering to an animal afflicted therewith a therapeutically effective amount of HFE2A modulator.

5. claims: 55-61

Method to diagnose individuals affected by or at risk of developing a disease of iron metabolism comprising determining a mutation or polymorphism in the nucleic acid sequence of the HFE2A gene.

6. claims: 67-83

Isolated polynucleotide comprising a polynucleotide having a nucleotide sequence with at least 60% identity to a sequence selected from the group consisting of Seq-ID-N° 1-9, 19-22; isolated polypeptide encoded by said sequences having at least 90% identity to an amino acid sequence selected from the group consisting of Seq-ID-N° 11-12, 23-28; cell line comprising a recombinant form of said polynucleotide or polypeptide, use of said cell line, composition for treating a disease comprising said polypeptide, method for treating a disease comprising administering said composition.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Although claims 33-54, 82, 83 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box II.1

Claims Nos.: 33-37, 44-48, 50-54, 84-85 (completely) 38-43, 49 (partially)

Claims 84-85: Rule 39.1(v) PCT - Presentation of information

Continuation of Box II.2

Claims Nos.: 33-37, 44-48, 50-54 (completely), 38-43, 49 (partially)

Present claims 33-37, 44-48, 50-54 (completely) and 38-43, 49 (partially) relate to an extremely large number of possible compounds and agents. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of said compounds and agents. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the antibodies, , anti-sense molecules and ribozymes.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 02051438	A	04-07-2002	WO	02051438 A2	04-07-2002
			EP	1355666 A2	29-10-2003
			JP	2004525875 T	26-08-2004
			US	2004102376 A1	27-05-2004
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